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13. ABSTRACT *(Maximum 200 words)*

Block copolymers of hydrophobic and hydrophilic acrylates were to be made and tested for control of mineralization by calcium oxalate. Acrylate copolymers in solution inhibited crystal growth to an extent dependent on their acid content but not dependent on the architecture. Solid films of acrylate polymers became mineralized when exposed to oxalate solutions. This effect was very dependent on the structure of the polymer. Methods were also developed for forming inorganic layers on polymer films by overlaying two polymers, each containing solubilized precipitants. Reaction and precipitation would occur at the film/film interface.

Cross-linked methacrylic acid films could be templated with rigid molecules such as caffeine, and then would be absorb the templated molecule selectively from solution. Methods were also developed for coupling short protein chains to acrylate polymers as a route to combining biochemical specificity with localization on inorganic surfaces.

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INTELLIGENT SYNTHETIC POLYMERS:
Final report to the Army Research Office

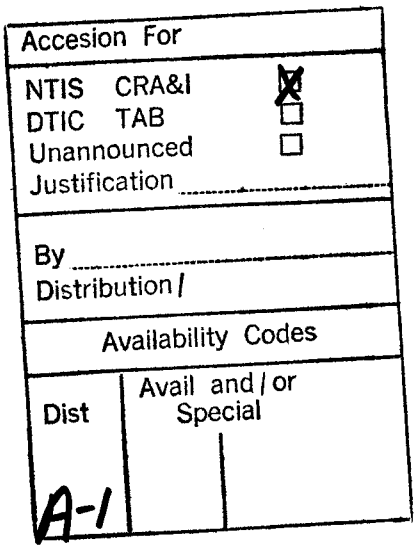
P.I.'s: Paul Calvert, Materials Science and Engineering, and H.K. Hall Jr, Chemistry, University of Arizona

Grant Number: DAAL03-91-G-0169 1991-1994

FOREWORD

The work described here is step on the journey to a synthetic world which is at least as subtle, varied and versatile as the natural world. Here we have exploited new methods of polymerization allow us to control polymer structure and produce molecules that are a little like proteins. This control of polymer architecture should let us mimic biomineralization, biological transport processes and ultimately biological catalysis. Before we can do this we not only have to have the polymerization methods but we have to understand the design rules.

We would like to thank David Kaplan of the U.S. Army laboratories in Natick for his help in setting up this project and for useful discussions throughout the last three years.



INTELLIGENT SYNTHETIC POLYMERS

BACKGROUND

Proteins are distinguished from synthetic polymers by their synthesis from a nucleic acid template which gives rise to a chain with an absolutely specified sequence of amino acids. Synthetic polymer chains are limited to simple repeats of a single unit, random copolymers or two or more units or blocks of two types of unit. This has not limited our ability to produce polymers with mechanical properties similar to those of structural proteins such as collagen or keratin. However it is not clear that we can make synthetic enzymes or transport proteins without detailed control of the chain sequence.

New methods for block polymerization of acrylic polymers give us more flexibility. Group Transfer Polymerization (GTP), developed by Sogah at DuPont, and anionic polymerization of acrylates, developed by Teyssie in Liege, both allow a wide range of acrylate monomers to be block copolymerized. This still only allows simple sequences of two or three blocks to be made but it does give access to a wide range of possible chain units, analogous to the variety of acidic, basic, polar and non-polar amino acids. Thus we still cannot approach the complexity of proteins but this is definitely a step forward.

We can now explore what special properties can be built into polymers using these blocks with controlled functionality. The work done under this grant built on two earlier projects. In one, which was motivated by a concern with ceramic processing, a series of block copolymers of acrylates were made by Group Transfer Polymerization (GTP) and tested as dispersants for alumina particles in solvents (1). GTP is a newly developed polymerization method which allows block copolymers to be made from polar monomers. This gives us the ability to make polymer chains where some sections interact strongly with inorganic surfaces while other sections are soluble in non-polar solvents.

A second area of previous work concerned the growth on inorganic particles in polymers. This research was stimulated by a desire to duplicate the composite structure of bone where the reinforcing hydroxyapatite grows within the tough collagen matrix. We had formed composites with inorganic oxides formed from soluble alkoxides by an in situ reaction in the polymer. One aim of the new project was to extend this to crystalline precipitates such as calcium carbonate and hydroxyapatite.

GOALS OF THIS PROJECT

Biomineralization and membrane transport were selected as two phenomena where the controlling effects of proteins were more likely to be due to the architecture of hydrophilic and hydrophobic blocks than to local site geometry. Block copolymers of hydrophobic and hydrophilic acrylates were to be made and tested. Methods were also to be developed for coupling short protein chains to acrylate polymers. The protein part of the hybrid molecule would then give the specificity while the acrylate could be used to anchor the chain.

RESULTS

Biomimetic Mineralization

There is general agreement that biological mineralization depends on close control of the inorganic precipitation by the organic phase. This results in control of particle size, shape and alignment which must be responsible for the good mechanical properties of mineralized tissues. There is less agreement on the mechanism of this control. Initially it was thought that mineralizing proteins carried anionic carboxylate or phosphate groups with a spacing that matches that of the metal ions in one crystal face of the mineral. The metal ions bind to the protein with the correct spacing to nucleate a crystal. There has been shown to be a close match between the spacing of ions along a protein chain in the β-sheet structure and the calcium ions in one face of aragonite. However the protein structure does not seem to vary with the mineral being formed and the lattice match is limited to one direction.

A second theory is that the local geometry of negative charge centers in the anionic groups corresponds to the geometry of the anions in the crystal. As a result, polymer-bound cations are as exposed as the ions in a growing crystal surface and this promotes binding of free anions in the right conformation. Other factors which have been suggested to be important include binding and orientation of preformed nuclei, catalytic activity of the surface which forms local high concentrations of cations or anions or favorable nucleation conditions within the polymer surface rather than actually at the interface.

The work on the modification of calcium salt precipitation by block co-polyacrylates was carried out by Mualla Oner, a postdoctoral fellow and has been fully reported (2, 3). Calcium oxalate was selected as the precipitating system since there are fewer potentially confusing side-issues than with calcium carbonate and calcium phosphates. Butylmethacrylate-methacrylic acid copolymers were added in various proportions, in the ppm range, to a crystallizing solution of calcium oxalate. The effect of the copolymers on crystallization time was measured. We had anticipated that a critical number of acid groups in a sequence would be necessary to inhibit crystallization. In fact the main effect seen depended on the fraction of acid groups in the chain but not on the sequence of them.

Beyond this we produced a series of polymer gels and films to study their mineralization tendency in solution. The ideal is to deposit, on a substrate, a film which promotes crystal growth. Thus a metastable supersaturated solution will grow crystals in or on the film but not free in solution. The has been put forward by Mann as the key to biomineralization (4). We have found that some hydroxylated acrylate polymers will mineralize from a calcium oxalate solution (presented at the Materials Research Soc. Fall 1994). Rieke has similarly treated surfaces with self-assembled monolayers for mineralization by cadmium sulfide and iron oxide (5). While we can form mineral films on organic surfaces, we do not fully understand why. It is clear that Mann's original concept of lattice templating is wrong and that the important interactions are more at the level of individual ion binding.

Fully developed systems of this type could become the basis for a technology for device manufacture. Patterned films would mineralize locally with materials that are too temperature sensitive to withstand the conditions of silicon technology and give rise to a family of devices with better chemical and thermal sensitivity, closer to the biological senses.

Protein-Polymer Hybrids

Work has continued to develop methods for coupling anionically polymerized acrylates to polypeptides. This was taken to the point of coupling alanine ethyl ester to the end of polybutylacrylate. This is chemically interesting but alanine has no very interesting functions. Masataka Kubo has extended this work to coupling histidine to the end of the acrylate chain, with protection of the ring secondary amine (6). The chain makes the histidine soluble in a range of organic solvents. This polymer should have interesting properties as an interfacially-active binder of metal ions. We have 100 mg of the material for testing. Kubo also made acrylate chains with a phosphoserine end group which mimics the phosphated proteins involved in shell mineralization.

Sajiv Boggavarapu is studying membrane interactions with various organic compounds as part of a parallel ASSERT project. When he has reached the required level of competence, he will incorporate the histidine polymer into an acrylate film and test for copper binding on the surface and in the film. In addition he will carry out oxalate crystallization over surfaces of acrylates containing the coupled phosphoserine to monitor its effect as a nucleator.

The synthetic work has shown that acrylates can be polymerized anionically to form a narrow molecular weight distribution and coupled to simple peptides. This gives us hydrophilic or hydrophobic chains of quite uniform length with a peptide end group. For a simple amino acid or peptide, it is necessary to protect the acid end group when coupling the amino end to the acrylate. In the case of histidine it is necessary to protect both the acid function of the amino acid and the ring >NH group. There are two difficulties in extending this to a large arbitrary peptide. Firstly, all side chain >NH and -COOH must be protected. This can be done by artificial protein synthesis, given a supply of amino acids with protected side groups. Secondly, the reaction rate of the acrylate chain with the peptide chain will decrease rapidly as both chain lengths increase.

Given the above, we do have a general method to couple short synthetic peptides to acrylate chains of defined length.

Templating Membranes

This work is being carried out under the associated AASERT project. It has been known for some time that a cross-linked gel of methacrylic acid can be "templated" selectively to take up a molecule with multiple hydrogen-bonding sites, such as caffeine. Templating is achieved by carrying out the original polymerization in the presence of caffeine which is then extracted by a solvent treatment. It is believed that the resin bonds around the template so that a hole with the correct geometry remains after the template molecule is removed. This effect has only been demonstrated with bulk resin which is

granulated and tested by chromatography. The template molecule is more likely to attach to the resin and so passes more slowly through a chromatography column than do other similar molecules. This effect is a bit mysterious in that one would expect that, given sufficient mobility to allow the template molecules to diffuse out, the gel would rearrange on a molecular scale during the extraction.

Sajiv Boggavarapu has developed a method for templating films. This depends on using trimethoxysilylpropylmethacrylate to cross-link the imprinted gels by a hydrolytic reaction after film formation. Using spectroscopic studies of these films, we have compared absorption of theophylline and caffeine by templated films. Selectivity for the templating molecule by about a factor of two is seen, caffeine-templated films take up more caffeine than theophylline and vice versa. Controls on un-templated films are in progress.

This effect has considerable implications for selective detection and removal of organic compounds. We are planning to extend the work to templating cavities for crystal growth.

Polymer bilayer methods for patterned mineralization

One route to localize deposits of minerals in a polymer is to identify specific surface groupings that will promote mineralization from a solution. This has only proved to be possible if the solution conditions are very closely controlled. An effort by Calvert, carried out at Sandia National Labs (presented MRS meeting, Boston, Fall 1994), assessed different methods of putting one reagent into a polymer film reservoir and then reacting with a solution to cause precipitation. A most reliable method was to incorporate one reagent, such as cadmium salts, into a polymer film which was printed onto a substrate. This film was overprinted with a second polymer containing a second reagent, such as a sulfide source. On heating a film of cadmium sulfide grew at the interface. The second polymer was then removed by a selective solvent. This method offers a route to the controlled and patterned deposition of multiple layer devices using sequential printing steps and liquid treatments.

This moves us toward the goal of developing a family of VLSI techniques that could be used under ambient conditions with aqueous solutions and would let us incorporate multiple layers of sensitive organic and biological molecules into devices.

Future Work

Solid Freeform Fabrication (SFF) allows the deposition of a wide variety of materials to build functional parts. Our system comprises computer-driven syringes which deposit slurries, of particles dispersed in liquid monomers, in patterns under the control of a CAD program. As each layer is formed it is cured to a solid. Simultaneous deposition of several materials allows complex composites to be formed. This method has much in common with biological growth of materials in that composite parts can be formed layer by layer. Chemical synthesis of shapes becomes possible from reagents being diffused into each layer. The localized control of the process is similar in scale to cellular control and allows composition and properties to be varied on the 100 μm scale

throughout the part.

Bone, tooth and shell also deposit in a layerwise fashion. We have come to see that SFF methods will allow formation of synthetic mineralized materials. Previously we had not seen how to avoid the problem of large shrinkages and small volume fractions associated with any chemical transformation in a bulk material. By carrying out the chemical changes in each layer as it forms, the shrinkage can readily occur in the z-direction and the diffusion distances are all short. This also led us to realize that layers could be deposited under water as well as in air. Our deposition needle then plays much the same role as the interfacial cells in a mineralizing system. These control the structure of the composite on the millimeter scale, while structure on the scale of microns or less is determined by the physics and chemistry of the reagents.

The methods developed under the current grant will become part of the toolbox of deposition methods for SFF, allowing us to control local mineralization and to incorporate enzymatic sensors and transport polymers in soft solid materials for a range of devices and implements.

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Research Personnel Supported
Postdoctoral Fellows: Mualla Oner, Jeremy Burdon, Masataka Kubo,
Research Students: Sajiv Boggaravapu, William Mollberg (MS Degree Awarded),
Research Scientist: Anne Padias.

Manuscripts Submitted
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Presentations

In addition this work was presented in seminars at US Army Natick (twice), Wright Patterson AFB, Hoechst-Celanese, Dow Chemical Co., 3M, Sandia National Labs., Universities of Delaware, Minnesota and Cincinnati, Case Western Reserve University, Courtaulds Plc, Swedish Academy of Sciences.

No reportable inventions